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Patterns of pollen cross-allergenicity

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Knowledge of patterns of pollen cross-reactivity is crucial for diagnostics and especially for formulation of immunotherapy vaccines in times of diminishing availability of pollen extract constituents. As phylogenetic relationships have become better clarified, it becomes apparent that cross-reactivity does reflect taxonomy in the very great majority of cases. Contradictory observations of unexpected cross-reactivity between unrelated plants, sometimes remarkably distant ones, require explanation. There are many proteins, presumably performing vital functions, that are tightly preserved throughout the evolutionary tree from plants to animals, such as profilins, lipid transfer proteins, and pathogenesis-related proteins. These might function as panallergens. The small differences that exist between these ubiquitous proteins explain why these are frequently minor allergens not reacting in the majority of allergic sera. This review summarizes cross-reactivity studies with both crude pollen extracts and purified or recombinant allergenic proteins. The patterns of cross-allergenicity that emerge should be helpful in guiding both diagnostic and therapeutic decisions. (J Allergy Clin Immunol 2003;112:229-39.)

Key words: Allergen, cross-reactivity, immunotherapy, pollen, taxonomy, protein

This review will address cross-reactivity of pollen aeroallergens. Of what concern is cross-reactivity to the practicing allergist? Both diagnosis and therapy of inhalant allergy are impacted by such relationships. Positive skin test results to nonendemic plants might be explained by prior exposure in a mobile population or cross-reactivity with endemic plants. The decision to include such a nonendemic component in an allergen immunotherapy formulation depends on factors such as whether related plants provide adequate coverage. This becomes crucial with diminishing availability of plant extracts. As optimal allergen doses become clarified, cross-reactivity will impact on formulation. Ignorance of cross-reacting allergens will increase the likelihood of adverse reactions due to inadvertent dosing with greater amounts of the same allergen.

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Abbreviations used
CIE: Crossed immunoelectrophoresis
CRIE: Crossed radioimmunoelectrophoresis
P-K: Prausnitz-Küstner

As documented in reviews during the past 20 years, a great deal of additional information has become available both on pollen cross-allergenicity and on plant systematics.[1,2] Great strides have been made with amino acid sequencing, gene sequencing, and cloning of recombinant allergens. This review will attempt to incorporate some of those advances. Despite this recent expansion of knowledge, pollen cross-reactivity data remain fairly limited; studies have not been done on the majority of pollen extracts used in practice. There has been great interest, however, in the relationship between pollen sensitization and food allergy, especially as manifested in the oral allergy syndrome. Because of space constraints, linkages such as the mugwort-celery-spice syndrome will not be discussed in the present review.

POLLEN INTERRELATIONSHIPS

Cross-reactivity inferred by plant systematics depends on 2 premises. The first is that more closely related plants will have greater shared antigens. The second premise is that the accepted botanical classification indeed reflects phylogeny; that 2 plants in the same genus truly evolved from a common progenitor, 2 in the same family from a more distant ancestor, and so forth.[3] Plants in the same genus would be expected to have the greatest number of shared allergens, those in the same family perhaps fewer, and distantly related plants would be expected to show little cross-reactivity. This approach has been validated with some exceptions, the most notable being the presence of panallergens such as profilins and pathogenesis-related proteins. Older taxonomic schemes were based primarily on morphologic similarities, which are sometimes misleading because of evolutionary drift along both divergent and convergent paths. The taxonomy utilized here is that of Judd et al.[4] Supporting data are derived from morphology, embryology, palynology, and analysis of biochemical, chloroplast gene, chromosome, and DNA data. Taxons of any size, including orders or

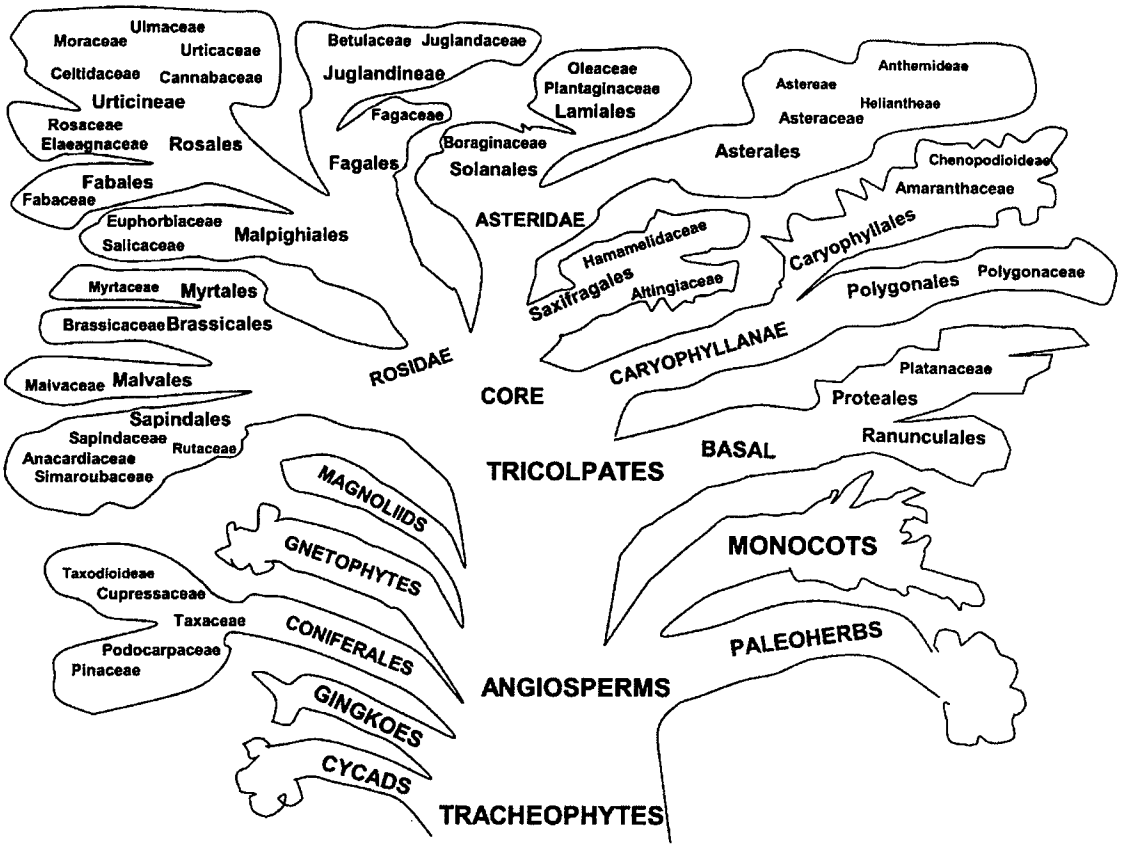

FIG 1. Taxonomy of vascular plants (subdivision Tracheophytina). Relationships are shown down to family or subfamily level. Not all orders and families are represented, only those with members demonstrated to be allergenic sources.

classes, related by a common ancestor are called clades. Where monophyly, or descent from a common ancestor, is very likely, one would expect strong cross-reactivity. However, significant controversy persists in placement of a number of plant groups.

Vascular plants, Tracheophytina, include spore-forming plants, such as ferns and club mosses, and seed plants. The latter group has traditionally been split into gymnosperms and angiosperms (flowering plants). Monophyly of angiosperms is strongly supported, but that of the gymnosperms is not. In the gymnosperms, cycads, gingkoes, and conifers are closely related, whereas the gnetophytes, containing *Ephedra* (Mormon tea), are more closely related to the angiosperms. The division of flowering plants into monocots and dicots is likewise not supported by recent data; monocots are monophyllous, but dicots are not. A large monophyllous subset is the Tricolpates, also known as true dicots (Eudicots). This group is identified by pollen type (containing 3 apertures) and similarities of gene sequences[4] (Fig 1).

Conifers

A large family in the order Coniferales is Cupressaceae, which includes cedars, junipers, pfitzers (*Juniperus* spp, *Thuja* spp), and cypresses (*Cupressus* spp). A prior small family, Taxodiaceae, including bald cypress, redwoods, sequoias, and Japanese red cedar (*Cryptomeria japonica*), has now been incorporated into Cupressaceae (Fig 1). Members of Cupressaceae are strongly cross-reactive, with most studies demonstrating consistent cross-inhibition with both animal antisera and human IgE antibodies.[5-7] In 1975, Yoo et al[5] examined 10 Cupressaceae members plus *C japonica* and coast redwood (*Sequoia sempervirens*). They found strong cross-reactivity between cypress family members but little with the latter 2 species. Also the redwood and Japanese red cedar did not cross-react. However, Yaseuda et al[8] reported cross-allergenicity between *C japonica* and a cypress, hinoki white cedar (*Chaemcyparis obtusa*), on the basis of skin tests and RAST inhibition. Schwietz et al[9] investigated 12 members of Cupressaceae including the major tree allergen of south Texas, mountain cedar (*Juniperus ashei*) and Japanese red cedar (*C japonica*), as well as a pine family member, deodar cedar (*Cedrus deodora*), and an angiosperm, salt cedar (*Tamarix gallica*). They found strong cross-reactivity between the major mountain cedar allergen, a 40-kd glycoprotein, with homologous allergens in the other family members, as well as the 46-kd major allergen of Japanese red cedar. A num-

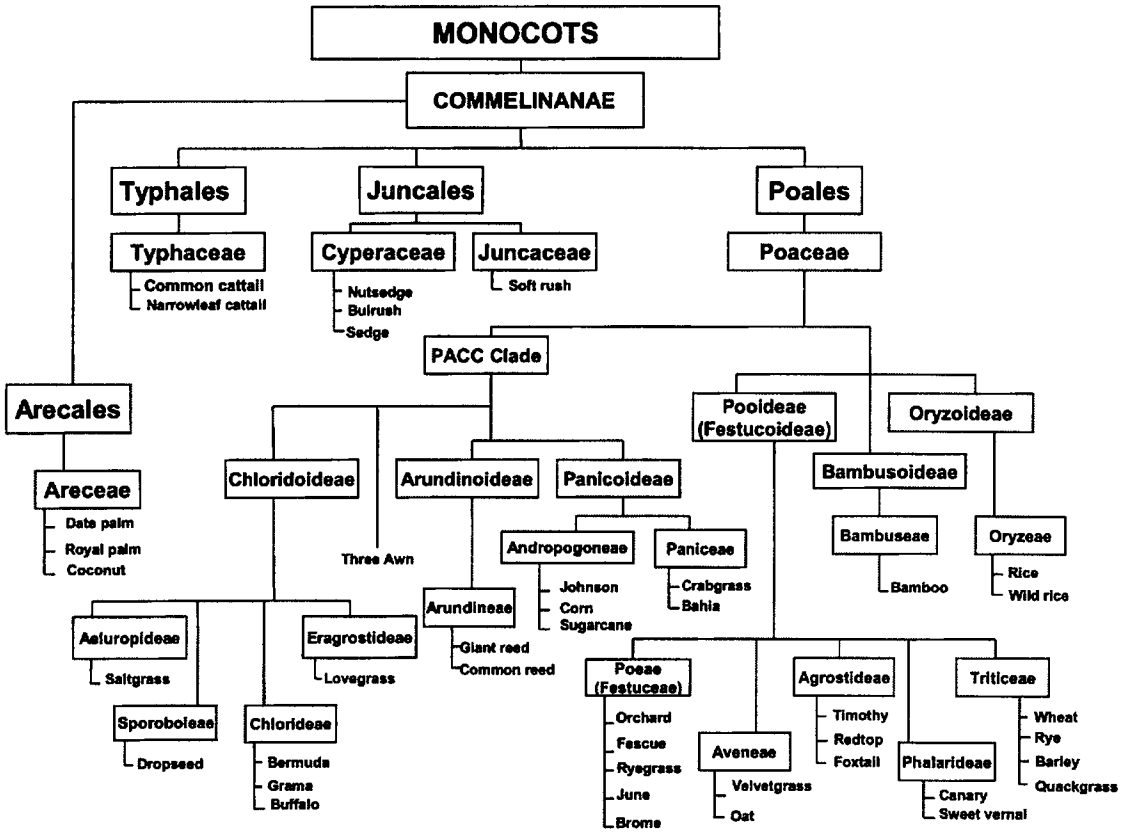

FIG 2. Monocot cladogram of allergenic members of superorder Commelinanae. Taxons of family, subfamily, and tribe level are depicted, with representative species by common name.

ber of allergens have now been cloned.[10,11] The amino acid sequences show high homology among group 1 allergens, but Jun a 1 contains N-glycosylation sites distinct from those of Cry j 1.[10,12] Tinghino et al[13] have reported an allergen from *J oxycedrus* (initially Jun o 2, now called Jun o 4), that belongs to a group of ubiquitous calcium-binding proteins. Immunoblotting studies showed inhibition by other family members: *J ashei*, *C arizonica*, and *C sempervirens*. The unrelated weed pellitory, *Parietaria judaica*, inhibited at low concentrations, whereas olive, *Olea europaea*, and ryegrass, *Lolium perenne*, inhibited at greater concentrations.

The pine family, Pinaceae, contains pines (*Pinus* spp), spruces (*Picea* spp), hemlocks (*Tsuga* spp), and firs (*Abies* spp, *Pseudotsuga*). Some rapidly eluted proteins from Monterey pine (*Pinus radiata*) are able to modestly inhibit solid phase perennial ryegrass (*Lolium perenne*) RAST discs.[14] While initially perplexing, this cross-reactivity, however modest, probably reflects panallergens such as the profilins.

Grasses

The class Angiospermopsida contains 2 major subgroups, the Monocots and Dicots, as well as additional lesser groups (Fig 1). The Monocot subclass has the lily superorder, Lilianae, and the spiderwort superorder, Commelinanae. A large clade within this latter superorder includes the grass family, Poaceae, and also rushes, sedges, and cattails (Juncaceae, Cyperaceae, and Typhaceae families, respectively). Poaceae is a large family with several subfamilies and numerous tribes. The subfamily Pooideae (Festucoideae) of temperate pasture grasses contains 5 tribes: Poeae, Aveneae, Phalarideae, Agrostideae, and Triticeae. The "PACC" clade contains the subfamilies Arundinoideae, Panicoideae, and Chloridoideae and grasses of the genus *Aristida* (three-awns). Panicoideae contains the tribes Paniceae and Andropogoneae. Chloridoideae has 4 tribes: Aeluropideae, Eragrostideae, Sporoboleae, and Chlorideae. Fig 2 depicts major taxons with representative grass species.

Several crude extract RAST inhibition studies of the fescue subfamily demonstrated that members strongly cross-react.[15-17] Bermuda appeared to have distinct allergens, as did timothy and possibly sweet vernal.[15,16] Martin et al[17] investigated a variety of grasses, finding similar results with the temperate pasture grasses, with western wheatgrass and quackgrass also inhibited by timothy and fescue. Bermuda, grama, and salt grass were cross-inhibitory, with Bermuda most potent. Johnson and Bahia grasses were

TABLE I. Purified grass pollen allergens

Allergen	Molecular weight (kd)	% Patient reactivity	Comments
Group 1	27-32	95	Papain-like cysteine protease (β-expansin)
Group 2/3	19-20	60	
Group 4	50-60	70	Isoelectric point = 9.0-9.3 carbohydrate-rich
Group 5 (old Grp 9)	27-33	65-85	
Group 6	10-15	60-70	
Group 7	8-12	10	Calcium-binding protein
Group 10	30		Cytochrome C (? relevance)
Group 11	16	>65	Soybean trypsin inhibitor–related
Group 12	14	20	Profilin
Group 13	55-60	50	Polygalacturonase

See Reference 22.

TABLE II. Grass pollen interrelationships

Taxon	Examples	Comments
Pooideae	Timothy, orchard, fescue, ryegrass, june, sweet vernal	1. Strong cross-allergenicity based on marked homology of group 1, 2/3, 5 major allergens 2. Possible unique allergens in timothy and sweet vernal
Chloridoideae	Bermuda, buffalo, salt, grama	1. Cross-reactivity between members, Bermuda strongest inhibitor 2. Lack of group 2 and 5 allergens accounts for little cross-allergenicity with Pooideae
Panicoideae	Johnson, corn, Bahia, sugarcane	1. Lack of group 2 and 5 allergens accounts for little cross-allergenicity with Pooideae 2. More cross-reactivity with Pooideae than Chloridoideae
Juncaceae, Cyperaceae, Typhaceae, Areceae	Rush, sedge, cattail, palm	1. Cross-allergenicity within families 2. No cross-reactivity with Poaceae

TABLE III. Tree and weeds interrelationships

Taxon	Examples	Comments
Coniferales	Cedar, juniper, mountain cedar, cypress, Japanese red cedar, redwood, pine, fir	1. Strong cross-allergenicity within Cupressaceae based on marked homology of group 1 and 2 major allergens 2. Weak cross-allergenicity with angiosperms might be due to group 4 calcium-binding proteins
Amaranthaceae	Palmer's amaranth, pigweed, Russian thistle, burning bush, saltbush, wing scale	1. Strong cross-allergenicity between amaranths 2. Strong cross-allergenicity between *Atriplex* species 3. Greater diversity, with variable degrees of reactivity with Chenopodioideae members 4. Russian thistle might possess unique allergens
Fagales	Birch, alder, hazel, oak	1. Strong cross-allergenicity between Betulaceae members based on homology of group 1 and 2 allergens 2. Fairly strong cross-allergenicity between Betulaceae and Fagaceae members based on homology of group 1 and 2 allergens
Oleaceae	Olive, ash, privet	1. Strong cross-allergenicity between Betulaceae members based on homology of group 1 allergens 2. Group 3 calcium-binding proteins cross-react
Asteraceae	Ragweed, mugwort, marshelder	1. Strong cross-allergenicity between short, giant, western, and false ragweeds 2. Strong cross-allergenicity between *Artemisia* species 3. Minor to little cross-reactivity between ragweeds and mugwort, marshelder, or cocklebur

inhibited by the northern grasses but were poor inhibitors. González et al[18] showed minor allergen cross-reactivity between Bermuda and other grasses by using RAST inhibition, immunoblots, and crossed radioimmunoelectrophoresis (CRIE). Cereal rye, wheat, and barley pollens showed similar levels of weak inhibition of grass pollen, whereas oat appeared somewhat different, and corn showed little cross-reactivity.[19] Rice pollen showed no cross-reactivity with corn pollen and weak cross-allergenicity with wheat, orchard, and timothy pollens.[20]

Beginning in 1966, Marsh et al[21] isolated 4 groups of antigens from perennial ryegrass, on the basis of molecular weight and isoelectric motility. Ten allergen groups have now been described (Table I).[22] Major grass aller-

gens are considered groups 1, 2/3, and 5, although reactivity to group 4 allergens has been reported in up to 75% of patients with grass allergy.[23] Group 1 allergens are β-expansins, papain-related cysteine proteinases, catalyzing long-term extension of plant cell walls.[24]

Intragroup epitope homology has been demonstrated between grass species and also between groups 1 and 2/3.[25-27] van Ree et al[27] demonstrated cross-reactive human and rabbit antibodies between groups 1 and 2/3 on the basis of homologies within the C-termini of Lol p 1, 2, and 3. By using a battery of mAbs to purified group 1 antigens, Esch and Klapper[28] showed cross-reactive epitopes as well as unique specificities. Groups 4 and 5 do not share cross-reactive epitopes.[29] Although some epitopes appeared to be shared by all grasses examined, others were not. Epitopes found on the timothy allergen Phl p 5 are shared with ryegrass group 1 allergens.[30] Matthiesen et al[31] demonstrated homology between the N-terminal sequences of Cyn d 1 and Lol p 1. By using mAbs and polyclonal antibodies, Chang et al[32] found cross-reactivity between Lol p 1 and minor allergens of Bermuda, but not with Cyn d 1. Smith et al[33] reported that although an mAb directed against the C-terminal of Lol p 1 did not bind the group 1 homologue of Bermuda or oat, they did find group 1 allergens in both the Pooideae and Panicoideae. They did not find group 5 allergens in other than the Pooideae subfamily, and some epitopes were not shared outside of the tribe Poeae.

Group 7 calcium-binding proteins are minor allergens, causing reactivity in 10% of patients with grass pollen sensitivity. Calcium-binding sites are called EF hands, and pollen allergens have been described with 2, 3, or 4 EF hands.[34] Two EF hand proteins are most common in grass allergens but are seen in other pollens as well: Phl p 7, Cyn d 7, Bet v 4, Aln g 4, Ole e 3, and Bra r 1. The timothy allergen, Phl p 7, showed the greatest degree of cross-reactivity.[34] Calcium-induced conformational changes in the molecule give rise to different IgE antibodies.[35] Bermuda grass Cyn d 7 shows sequence similarity with birch Bet v 4 and oilseed rape Bra r 1, with IgE cross-reactivity shown with the latter allergen.[36]

Ekramoddoulah and associates characterized cytochrome C as group 10 allergen, but further work has not substantiated it as a relevant allergen.[22] Lol p 11 is a 16-kd glycoprotein with 32% homology with soybean trypsin inhibitor, without the enzymatically active site.[37] There is no homology with other described grass allergens, but a specific mAb did bind with *Dactylis glomerata*, *Festuca rubra*, and *Phleum pratense*. However, similarity was found with corn and tomato pollen proteins and 44% homology with the major olive allergen, Ole e 1. Recombinant Bermuda grass profilin, Cyn d 12, shows equal IgE reactivity with natural Bermuda grass allergen and shares B-epitopes with sunflower profilin.[38] Niederberger et al[39] compared crude extracts with preabsorption with recombinant timothy Phl p 1, Phl p 2, Phl p 5, and birch profilin Bet v 2. The human sera pool was comprised of European, American, and Asian subjects. IgE to the 4 recombinant proteins accounted for a mean 59% of grass pollen–specific IgE. Lower inhibition of IgE binding to the crude extracts of Bermuda grass and corn, *Zea mays*, was attributed to the absence of detectable group 2 and 5 in these 2 species.

Tricolpate angiosperms

Three subclades within the tricolpate clade contain the majority of plants incriminated in pollinosis: the subclasses Asteridae and Rosidae and the superorder Caryophyllanae (Fig 1). The latter taxon includes the orders Caryophyllales and Polygonales. Older classifications had chenopod and amaranth weeds in separate families in the order Chenopodiales. Chloroplast DNA restriction sites, ribulose-1,5-bisphosphate carboxylase/oxygenase (*rbcL*) sequences, and morphology evidence suggest inclusion within Caryophyllales, and the chenopod weeds are now contained in the Chenopodioideae subfamily within the Amaranthaceae family.[4] Few cross-reactivity data are available for Polygonaceae. A Prausnitz-Küstner (P-K) study of sheep sorrel (*Rumex acetosella*) extract showed strong but incomplete suppression of the sheep sorrel sites by ragweed, plantain, and timothy but not the reverse, suggesting relevant allergens of sheep sorrel are minor allergens in the other plants, as well as a minor unique allergen.[40]

The chenopod-amaranth weeds contain major inducers of pollinosis in the western and Great Plains states, including tumbleweeds (*Salsola* and *Kochia*), scales (*Atriplex* spp), and pigweeds (*Amaranthus* spp). Sellers and Adamson[41] used P-K extinction to compare 4 *Amaranthus* species with lamb's quarters (*Chenopodium album*) and Russian thistle (*Salsola pestifer*). There was complete inhibition between the amaranth extracts as well as the 2 chenopods, whereas chenopods could not inhibit amaranths. Russian thistle was more effective in inhibiting lamb's quarter. Wodehouse[42] used Ouchterlony plates to examine the cross-antigenicity of specific Russian thistle and Palmer's amaranth rabbit antisera against 2 *Atriplex* weeds, 3 other chenopods, and 4 amaranths. The amaranths showed basically identical precipitin bands; similarly, the *Atriplex* weeds showed identity. Although the other chenopods were much more heterogeneous, Russian thistle and burning bush (*Kochia scoparia*) showed a common antigen with the amaranths. Weber[2] examined 9 chenopods (including 4 Atriplexes) and 3 amaranths by skin test correlation, passive double immunodiffusion with rabbit antisera, and RAST inhibition. Redroot pigweed, *A retroflexus*, and Palmer's amaranth, *A palmeri*, were identical by all techniques, with western water hemp, *Amaranthus* [*Acnida*] *tamariscina*, demonstrating fewer precipitin bands. The scales, *Atriplex* spp, showed similar RAST inhibition curves and the same antigen precipitin bands. Although containing the greatest number of cross-reacting antigens on immunodiffusion, lamb's quarter was a weak inhibitor on RAST. Russian thistle was a potent inhibitor on RAST and appeared to have unique allergens. Crossed immunoelectrophoresis (CIE), isoelectric focusing, and SDS-

PAGE immunoblots showed 5 and 12 antigens in these chenopod-amaranth weeds, ranging from 20 to 65 kd. Wurtzen et al[43] examined *A retroflexus, C album, K scoparia,* and *S pestifer* extracts with CIE, CRIE, and SDS-PAGE immunoblotting. The 3 techniques gave somewhat disparate results, possibly from denaturation in the SDS medium. Russian thistle appeared to have the greatest number of potent allergens. Two Russian thistle isoallergens of 30 and 42 kd have been isolated.[44] A major allergen from lamb's quarter, Che a 1, has been characterized as a 17-kd glycoprotein.[45] Despite showing 27% to 45% identity with members of the Ole e 1–like protein family, there was no significant IgE cross-reactivity between Che a 1 and Ole e 1. Only low level IgE or IgG cross-reactivity was demonstrated.

The 2 subclades of Rosidae have pollinosis-inducing members (Fig 1). The first subclade contains several orders with very important allergenic plants: Rosales, Fagales, Fabales, and Malpighiales. The legume family, Fabaceae, contains numerous shrubs and trees such as locust, *Robinia* spp, mesquite, *Prosopis juliflora,* and alfalfa, *Medicago sativa.* There are no cross-reactivity data available between family members. Reports of weak cross-reactivity between *Acacia* and grasses presumably reflect the presence of strongly conserved proteins such as profilins and pathogenesis-related proteins. Russian olive, *Elaeagnus angustifolia,* is in Elaeagnaceae and, by ELISA inhibition, cross-reacts with European olive, ash, and privet, members of Oleaceae (subclass Asteridae, order Lamiales).[46]

The suborder Urticineae, previously classified as the separate order Urticales, contains Ulmaceae, Moraceae, Urticaceae, and Cannabaceae. Hackberry, *Celtis* spp, used to be in Ulmaceae but is now thought to be more closely related to Urticaceae and has been placed in a separate family, Celtidaceae. Pellitory, *Parietaria* spp, is a major aeroallergenic plant in the Mediterranean basin. In a skin test comparison by Serafini,[47] pellitory reactivity was quite strong whereas nettle reactivity was weak, suggesting little cross-reactivity between these family members. Holgate et al[48] found similar discordance in British patients.

The order Malpighiales contains Euphorbiaceae and Salicaceae. The former family includes a number of plants with latex-like sap. The major allergen, Mer a 1, of annual mercury, *Mercurialis annua,* has been cloned and is a profilin.[49] The recombinant protein reacts with sera from patients allergic to annual mercury, olive (*Olea europaea*), and castor bean (*Ricinus communis*) pollens and shares B-epitopes with sunflower profilin. Latex profilin, from *Hevea brasiliensis,* is highly cross-reactive with ragweed profilin but does not appear to cross-react with annual mercury profilin.[50] Immunoblot studies by Fuchs et al[51] showed that ragweed, mugwort, and timothy extracts could inhibit IgE binding to latex allergens. However, Bet v 1 and Bet v 2 (birch profilin) did not appreciably inhibit the latex IgE binding.

Salicaceae contains cottonwoods, poplars, and aspens (*Populus* spp) and willows (*Salix* spp). Appearance of strong cross-reactivity within this family is based on skin test correlations and older work with P-K neutralization.[2,52] Moderate cross-reactivity between Salicaceae and Fagales members is based on P-K neutralization and passive hemagglutination inhibition.[2,52]

One branch of the order Fagales includes the beech family, Fagaceae, and another large branch is the Juglandineae suborder, including the birch family, Betulaceae and Juglandaceae. The latter contains walnut, *Juglans* spp, pecan and hickory, *Carya* spp. Skin test correlations showed very high *r* values between shagbark hickory, *Carya ovata,* and pecan, *Carya illinoensis,* and moderate correlations of these with black walnut, *Juglans nigra.*[2] Skin test correlations and direct RAST determinations supported the cross-reactivity of the beech and birch families.[2,53] P-K neutralization has shown complete cross-desensitization among oak species.[54] Rackemann and Wagner[52] showed that birch could completely desensitize oak, maple, and willow sites, but the converse was not true. Oak was able to desensitize most of maple and willow sites. There appeared to be varying patterns of shared allergens among the other trees studied. RAST inhibition studies by Bernstein et al[15] showed cross-inhibition between oaks and other Fagales members, and Zetterström et al[55] showed birch to be a potent inhibitor of other Fagales as well as ash, elm, and willow.

Extensive work has been done on purifying, characterizing, and sequencing major allergens of the birch family. N-terminal amino acid sequencing of the major alder allergen, Aln g 1, by Ipsen and Hansen[56] showed partial identity with the corresponding pollen allergens of birch, Bet v 1, hornbeam, Car b 1, and oak, Que a 1. A second allergen is Bet v 2, a profilin; both Bet v 1 and Bet v 2 have been cloned. Recombinant DNA studies showed strong homology between Bet v 1 and these other major allergens.[57] Cross-inhibition of IgE immunoblots by the mix of recombinant Bet v 1 and Bet v 2 has been reported; homologous inhibition was an average of 92%, whereas inhibition by alder, hornbeam, hazel, and oak was 88%, 77%, 80%, and 72%, respectively.[58] The mix accounted for an average of 82% of the specific IgE, most being directed against rBet v 1. Cross-reactivity between birch, mugwort, and celery has been demonstrated with Bet v 1, Bet v 2, and higher molecular weight allergens (46 to 60 kd).[59] A 24-kd calcium-binding protein has been identified as Bet v 3 and a smaller 9-kd calcium-binding protein as Bet v 4.[60,61] Bet v 2, Bet v 3, and Bet v 4 are allergenic for 10% to 20% of patients with birch sensitivity. Another allergen, Bet v 5, reactive in about 30% of patients, is an isoflavone reductase–related protein, with cross-reactivity to pear and lychee food allergens.[62] The group 4 calcium-binding protein from alder, Aln g 4, shares IgE epitopes with similar allergens in tree, grass, and weed pollens.[63] Recombinant Aln g 2 cross-reacts with an oilseed rape allergen.[64]

The second Rosidae subclade accommodates a diverse number of allergenic plants. Oilseed rape (*Brassica napus*) is a heavily cultivated turnip-like oil-plant. Welch

et al[65] examined cross-reactivity between grass and oilseed rape in co-sensitized individuals by using RAST inhibition and immunoblot inhibition. They found less than 10% RAST inhibition of solid phase oilseed rape by grass, and immunoblot studies supported the distinct character of the respective allergens. Oilseed rape pollen allergens have been identified with weights of 6 to 8 and 14 kd, as well as a cluster of higher molecular allergens from 27 to 69 kd. The first two are a calcium-binding protein homologous to Aln g 2 and a profilin, respectively. The larger allergens have IgE-binding carbohydrate moieties, which cross-inhibit timothy group 4 allergens.

Sapindales includes Rutaceae, Simaroubaceae, Sapindaceae, and Anacardiaceae. Maples and box elder (*Acer* spp), previously members of a separate 2-genera family, Aceraceae, are now incorporated into Sapindaceae. P-K neutralization data showed cross-inhibition between maple and Fagales members, but RAST inhibition with box elder solid phase showed poor inhibition with most trees tested and none with hazel, birch, or alder.[15,52]

The majority of the subclass Asteridae is divided between 2 groups. The first group contains the orders Lamiales and Solanales. The latter includes the common Australian weed Paterson's curse, *Echium plantagineum*, family Boraginaceae. Katelaris et al[66] and Baldo et al[67] have shown skin test, RAST, CIE, and CRIE similarities between it and English plantain, *Plantago lanceolata*, in the family Plantaginaceae, order Lamiales. Cross-reactivity might be due partially to cytochrome c allergens.[68] The work by Rackemann and Wagner[52] had shown that grass and ragweed extracts could neutralize plantain but not the reverse, suggesting relevant allergens of plantain are contained by grass and ragweed but not vice versa. The N-glycan moiety of the major plantain allergen, Pla l 1, does not appear to function as a cross-reacting carbohydrate determination.[69]

Oleaceae, containing both shrubs and trees such as ash and olive, is also in the order Lamiales. Strong cross-reactivity was demonstrated between family members by using RAST inhibition, isoelectric focusing, and tandem CIE, with olive generally being the strongest inhibitor.[70] Immunoblotting studies by Baldo et al[71] identified 3 major allergens of olive at 18 to 19, 20, and 40 kd, with an additional 70-kd allergen in privet.

At least 7 olive allergens have been completely or partially sequenced. Ole e 2 is a profilin, and Ole e 5 is a superoxide dismutase; Ole e 3 and Ole e 8 are calcium-binding proteins, 2 and 4 EF hand, respectively. Ole e 3 is an acidic 9.2-kd protein.[72] Obispo et al[73] raised mAbs against the 17 to 19 kd major allergen of olive, Ole e 1, and showed by CRIE and SDS-PAGE antigenic and allergenic epitope sharing between Ole 1, Fra e 1, Lig v 1, and Syr v 1. N-terminal sequencing showed identity of the first 20 amino acids between these allergens. Martin-Orozco et al[74] demonstrated that all 4 had 18 and 20 kd Ole e 1 isoallergen homologues, and forsythia had a 50 to 55 kd cross-reactive protein. Ole e 1 has a single glycosylation site, which is an IgE-binding epitope.[75] Antibodies directed against this glycan moiety cross-react with horseradish peroxidase, bromelain, and ascorbate oxidase. Study of the calcium-binding motifs of Ole e 3 and Ole e 8 did not show any significant IgE cross-reactivity.[76]

Wahl et al[77] could barely detect a Bet v 1 homologue in ash pollen but did show some common high molecular weight allergens between birch and ash. However, Niederberger et al[78] demonstrated a number of ash allergens: Fra e 1, the Ole e 1 homologue; a profilin homologue of Bet v 2; a 2 EF hand calcium-binding protein cross-reactive with Phl p 7; a 35-kd allergen cross-reactive with birch, ragweed, and olive allergens; and some high molecular weight components. As mentioned above, one study has shown strong cross-inhibition between olive, privet, and Russian olive, the latter tree in a different subclass (Rosidae).[46]

Asteraceae (Compositae) is a huge family with 3 subfamilies and 17 tribes. The subfamily Asteroideae is monophyletic and contains 10 tribes, of which 3 are of particular interest: the sunflower tribe, Heliantheae; aster tribe, Astereae; and the mayweed tribe, Anthemideae. Ragweeds, once in their own tribe Ambrosieae, currently are incorporated into a subtribe of Heliantheae, Ambrosiinae. There are numerous species, with 4 major ragweeds being short, *Ambrosia artemisiifolia*; giant, *A trifida*; western, *A psilostachya*; and false, *A acanthicarpa*. Other members of Ambrosiinae include poverty weed and marsh elder, *Iva* spp, and cocklebur, *Xanthium communis*. Anthemideae includes mugwort and other sages, *Artemisia* spp. Astereae contains goldenrod, *Solidago* spp, and *Baccharis* spp.

Allergenic identity of short and giant ragweeds was supported by skin test comparisons, P-K neutralization, and extract interchangeability during immunotherapy.[79-81] But others reported inadequacy of monospecies immunotherapy, skin test result disparities, incomplete P-K neutralization with whole as well as partially purified extracts, and nonidentity of precipitins on Ouchterlony immunodiffusion.[82-85]

Comparison of the 4 major ragweeds by RAST inhibition found them roughly equivalent, with false ragweed being the most effective inhibitor.[15] Leiferman et al[86] found overlapping RAST inhibition curves for short, giant, western, and false ragweeds; slender and southern ragweeds were less potent, and other members of the family showed no inhibition.

Prince and Secrest[87] showed by P-K neutralization that marsh elder was allergenically distinct from short, giant, and western ragweeds. Suzuki et al[88] demonstrated that neither ragweed nor goldenrod could desensitize chrysanthemum sites. Leiferman et al[86] showed with RAST inhibition that cocklebur (*Xanthium communis*) and annual wormwood (*Artemisia annua*) could weakly inhibit short ragweed, but that other sages (*Artemisia* spp) and burweed marsh elder (*Iva xanthifolia*) could not. RAST inhibition data between western ragweed and Santa Maria feverfew, *Parthenium hysterophorus*, suggested unique and cross-reactive allergens.[89] A study of short and giant ragweed and feverfew by using both US and Indian patient sera (to isolate primary sensitization to ragweeds and feverfew,

respectively) demonstrated marked cross-inhibition.[90] Examining pollens from several non-ragweed Asteraceae members, Løwenstein, by using crossed-line immunoelectrophoresis and CRIE, demonstrated 25% to 75% shared antigens and 0 to 2 shared allergens.[91]

At least 7 short ragweed allergens have been characterized or cloned, the most important being Amb a 1 (Ag E). Most have several isoforms, and there are so many naturally occurring isoelectric forms of Amb a 1 that it should be considered a family of closely related proteins rather than a single molecule.[92,93] Differing recombinant forms of Amb a 1 can elicit distinct reactions at both T- and B-cell levels.[94] Amb a 1 and Amb a 2 (Ag K) are 38-kd acidic multi-chain molecules with several shared IgE binding epitopes as well as unique sites.[95-98] Most clinically sensitive ragweed sufferers will react to Amb a 1 and Amb a 2 (~90% to 95%); between 10% to 21% will respond to the lower molecular weight basic proteins Amb a 3, Amb a 5, and Amb a 6.[99] Certain patients are able to distinguish between the homologous allergens from short and giant ragweed, Amb a 5 and Amb t 5.[100] T-cell epitope mapping of Amb a 5 and Amb t 5 has demonstrated the importance of reduction of disulfide bridges into free sulfhyfyl groups to elicit T-cell hydridoma cross-reactivity between the 2 related allergens.[101] Yunginger and Gleich,[102] by using double antibody radioimmunoassay, found Amb a 1 in short, giant, southern, and false ragweed, with minimal amounts in slender ragweed. No Amb a 1 was found in cocklebur and marsh elder (same subtribe), sages and mugwort (different tribe), plantain (different order), or a variety of more distantly related angiosperms. Lee and Dickinson[103] found Amb a 1 in decreasing amounts in pollen extracts from short, western, southern, canyon, slender, giant, and false ragweeds, respectively. They also found Amb a 1 in cosmos (same tribe) but not in sunflower, narrow-leaf marsh elder, or spiny cocklebur. Krilis et al,[104] by using mAb-based enzyme immunoassay, found Amb a 1 in a commercial ragweed mix, lesser amounts in an experimental short ragweed extract, but not in a similar false ragweed extract or in mugwort, goldenrod, Paterson's curse, timothy, plantain, and perennial ryegrass extracts.

By using ELISA inhibition and immunoblot inhibition, Katial et al[105] examined cross-reactivity in 9 *Artemisia* species. They found strongly overlapping inhibition curves, with *A tridentata* and *A biennis* appearing slightly more potent. Inhibition of immunoblots was virtually complete among the 9 species. Brandys et al[106] studied giant sagebrush, *A tridentata*, as well as 5 European and Asian sages. They also showed strong cross-reactivity and, in addition, thought that there was greater similarity between allergens of greater than 25 kd and more heterogeneity among allergens less than 25 kd. White wall rocket, *Diplotaxis erucoides*, family Brassicaceae, shows enzyme immunoassay cross-inhibition with mugwort.

Two mugwort allergens have been identified: Art v 1, a 60-kd glycoprotein, and Art v 2, a 35-kd glycoprotein.[107,108] Hirschwehr et al[109] demonstrated, by using sera of patients with mugwort sensitivity, that the major allergen Art v 1 was shared by short ragweed. Cross-reactivity with birch profilin Bet v 2 was also found. Mugwort profilin has been recently characterized.[110]

Thirteen allergens were detected in *Helianthus annua* pollen extract by Fernandez et al[111] on immunoblots, with two of 24 to 25 kd weight reacting with 95% to 100% of the patient sera. RAST inhibition and immunoblot inhibition showed varying degrees of cross-reactivity, with mugwort the greatest, followed by oxeye daisy (marguerite), *Chrysanthemum leucanthemum*, dandelion, *Taraxacum vulgare*, goldenrod, *Solidago vigaurea*, and short ragweed the least. A strongly cross-reactive profilin has been identified as Hel a 2.[112]

CONCLUSIONS

Refinement of plant systematics with clarification of phylogenetic relationships has demonstrated that the basic premises entertained hold true: cross-allergenicity does reflect taxonomy in the very great majority of cases. The contradictory observations of unexpected cross-reactivity between very distantly related plants fly in the face of the basic premise mentioned above, that taxonomy dictates cross-reactivity. Advances in gene sequencing and the cloning of recombinant proteins have led to the understanding that there are many tightly preserved proteins, even from plants to animals, such as profilins, lipid transfer proteins, and pathogenesis-related proteins. The small differences that exist between these ubiquitous proteins, which presumably perform vital functions, explain why these are frequently only minor allergens.

Conifers

Cupressaceae members are markedly cross-reactive. A single *Juniperus* pollen extract should be suitable for immunotherapy. Members of Pinaceae should be treated separately in the rare instance of clinical importance. There is no significant cross-reactivity between conifers and flowering plants; the minor reactivity is not of clinical relevance.

Grasses and related plants

Members of the grass subfamily Pooideae are strongly cross-reactive on the basis of marked homology of groups 1, 2/3, 4, and 5, most of which are major allergens. Differences in cross-inhibition probably reflect quantitative differences rather than qualitative ones, although it is possible that timothy and sweet vernal might contain relevant unique allergens. In testing with and immunotherapy for northern pasture grasses, adequate coverage should be achieved by 1 or 2 members, and there is no need for multiple representation. The Panicoideae and Chloridoideae subfamilies show greater diversity and lack group 2 and group 5 allergens, accounting for their differences with the temperate grasses. Members should be tested for and treated with separately. Chloridoideae members appear to be cross-reactive, with Bermuda grass being very potent and the

appropriate choice to cover other members. The cross-reacting profilins and calcium-binding allergens are minor allergens. Other non-grass families of monocots show reactivity between members but no appreciable cross-reactivity with grasses (Table II).

Tricolpate angiosperms

Diversity is the rule among the plants in these subclasses and orders. Lack of cross-reactivity is the rule even down sometimes to the level of tribe. There are, however, exceptions with significant cross-reactivity across families (Table III).

In Amaranthaceae, allergenic identity is almost complete among the *Amaranthus* species such as redroot pigweed and Palmer's amaranth and slightly less with another member, western water hemp. In the Chenopodioideae, there is greater diversity, although there are striking similarities among the *Atriplex* saltbushes and scales. There are varying degrees of cross-inhibition between other chenopods and amaranths, and Russian thistle appears to possess significant unique allergens. *Amaranthus* and *Atriplex* species can be represented by single members, but the other locally relevant members need to be addressed separately.

The order Fagales shows strong cross-allergenicity within the birch family, extending across to the beech family as well. Use of the locally prevalent Betulaceae member should cover other family members as well. In areas where oaks are predominant, a *Quercus* would be expected to cover birch as well. Immunotherapy with rBet v 1 and rBet v 2 recombinant allergens could effectively cover Fagales tree pollen sensitivity. There is scant information concerning other members of the Rosidae subclass. Generally, one member of a family can be expected to be adequate for immunotherapy, although there are exceptions such as nettle and pellitory.

Olive family members are strongly cross-reactive. In areas where European olive is grown, it seems the proper choice. In other areas where ash is prevalent, it should be adequate. Treating with multiple members should not be necessary.

Asteraceae members show variable cross-reactivity. The 4 major ragweeds (short, giant, western, and false) strongly cross-react, and 1 or 2 are quite adequate. However, in areas where the sages or marsh elders are common, these need to be handled separately. Because *Artemisia* species are so strongly cross-reactive, it is not necessary to skin test or treat with multiple members; a single choice will do. In the Midwest and West common sagebrush (*A tridentata*) seems reasonable, whereas in the eastern states and Europe, mugwort (*A vulgaris*) seems most appropriate.

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